incubation. After digestion, the reactions were size fractionated using the appropriate agarose gel depending on the assay specifications (2.5%, 3%, or metaphor). Gels are electrophoresed in 1X TBE Buffer at 170 Volts for approximately two hours.

The gel is illuminated using ultraviolet light and the image is saved as a Kodak 1D file. Using the Kodak 1D image analysis software, the images are scored and the data is exported to EXCEL.

2. ASO assay. The amplicon, containing the polymorphism, was PCR amplified using primers that were used to generate a fragment for sequencing (sequencing primers) or SSCP (SSCP primers). The appropriate population of individuals was PCR amplified in 96 well microtitre plates and re-arrayed into 384 well microtitre plates using a Tecan Genesis RSP200. The amplified products were loaded onto 2% agarose gels and size fractionated at 150V for 5 minutes. The DNA was transferred from the gel to Hybond N+ nylon membrane (Amersham-Pharmacia) using a Vacuum blotter (Bio-Rad). The filter containing the blotted PCR products was transferred to a dish containing 300mls of pre-hybridization solution (5x SSPE {pH7.4}, 2% SDS, 5x Denhardts). The filter was left in the pre-hybridization solution at 40°C for >1 hour. After pre-hybridization, 10mls of the pre-hybridization solution and the filter were transferred to a washed glass bottle. The allele specific oligonucleotides (ASO) were designed with the polymorphism in the middle. The size of the oligonucleotide was dependent upon the GC content of the sequence around the polymorphism. Those ASOs that had a G or C polymorphism were designed so that the Tm was between 54-56°C and those that had an A or T variance were designed so that the Tm was between 60-64°C. All oligonucleotides were phosphate free at the 5'end and purchased from Gibco BRL. For each polymorphism 2 ASOs were designed: one for each variant.

The two ASOs that represented the polymorphism were resuspended at a concentration of 1 μg/μl and separately end-labeled with γ-ATP^{32} (6000Ci/mmol) (NEN) using T4 polynucleotide kinase according to manufacturer recommendations (NEB). The